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PHARMACOKINETICS AND BIOAVAILABILITY OF MIDAZOLAM IN MAN

P. HEIZMANN, M. ECKERT & W.H. ZIEGLER*

F. Hoffmann-La Roche & Co. Ltd, CH-4002 Basle and *Division of Clinical Pharmacology, Department of Medicine, University Hospital, CH-8091 Zürich, Switzerland

1 The pharmacokinetic behaviour and the bioavailability of midazolam were investigated in six volunteers after intravenous (0.15 mg/kg) and oral administration (10, 20 and 40 mg).

2 Following rapid *intravenous injection* of midazolam, the plasma concentration of the substance decreased to approximately 10% within 2 h owing to a rapid rate of distribution.

3 A two compartment model adequately described the kinetics of midazolam in plasma. The following average values were found: elimination half-life, 2.3 h; total clearance, 323 ml/min, and apparent volume of distribution at steady-state (V_{ss}), 50.2 l.

4 After *oral administration*, the drug is rapidly absorbed. Maximum plasma levels are reached within 30 min and the drug is rapidly eliminated from plasma with practically the same half-life as determined after i.v. administration.

5 The bioavailability after the ingestion of 10, 20 and 40 mg midazolam in the form of tablets ranged from 31 to 72%, due to the high liver extraction quota of midazolam.

Introduction

Midazolam belongs to a class of benzodiazepine derivatives which are characterized by rapid onset and short duration of pharmacodynamic effects. The salts of midazolam are water-soluble and are stable in aqueous solution.

Midazolam is rapidly eliminated from the body, almost exclusively by metabolic processes (Figure 1) (Heizmann & Ziegler, 1981). Elimination half-lives after intravenous infusions are reported to be in the range 1.3–3.1 h (Smith *et al.*, 1981), 1.3–2.2 h (Puglisi *et al.*, 1978) and 2.1–3.4 h (Greenblatt *et al.*, 1981).

After oral administration of ^{14}C midazolam to human subjects (Heizmann & Ziegler, 1981) about 90% of the given radioactivity appears in the urine within 24 h, three-quarters of the dose occurring in the form of the conjugated α-hydroxy-metabolite. This metabolite is pharmacologically active in animals but markedly less than unaltered midazolam. Clinically, this metabolite is of no significance as an active component since, immediately after its formation, it is conjugated by glucuronic acid to form a pharmacologically inactive end product.

Two further metabolites, 4-hydroxy-midazolam and α,4-dihydroxy-midazolam, are excreted in insignificant amounts as conjugates in the urine (3% and 1% of dose, respectively). No measurable amounts

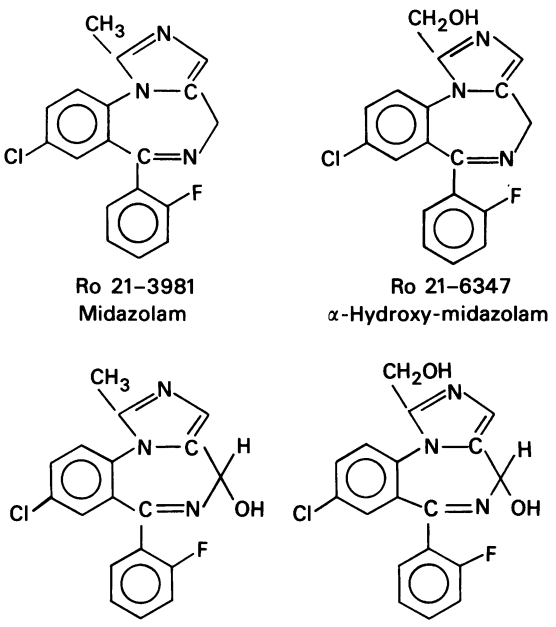

Figure 1 Metabolites detectable in the urine after oral administration of midazolam.

of unchanged drug are excreted in the urine (Heizmann & Ziegler, 1981).

The present study was performed to elucidate the pharmacokinetic behaviour of midazolam following intravenous and oral dose administration and to calculate its bioavailability.

Methods

Experimental design

Six normal healthy volunteers were used for the study. All were of similar age (22–27 years) and weight (55–77 kg). The experimental protocol was approved by the area Ethical Committee and all subjects gave written, informed consent before commencing the study.

They were instructed that no drug should be taken two weeks prior to the date of each administration and that no alcohol was permitted over the entire study period. A two-week medication-free interval was observed between the individual administrations. Food and fluid intake was standardized on study days.

Each volunteer received three single doses of different preparations. A single intravenous dose of 0.15 mg/kg and an oral dose of 20 mg in the form of two tablets was administered. For the third administration, four of the volunteers received 10 mg midazolam orally and two subjects received an oral dose of 40 mg. The oral dose was given on an empty stomach in the morning 1 h before breakfast.

Sample collection

Blood samples (7 ml into vacutainers) were withdrawn through an indwelling cannula previously inserted into an antecubital vein, at the following times: pre-dose (control), 15, 30, 45, 60 and 90 min and at 2, 3, 4, 5, 6, 8, 10 and 12 h after oral administration. Two additional samples were taken at 5 and 10 min after intravenous injection of the drug. Oxalates were used as anticoagulants for the preparation of the plasma. Prior to analysis, all plasma samples were stored at −20°C. In both intravenous and oral experiments, urine was collected at the following times: pre-dose (control), 0–6, 6–12, 12–24 h after drug administration. Total volumes were measured and aliquots subsequently stored at −20°C until required for analysis.

Assay procedure

Plasma Plasma samples were assayed for unchanged midazolam and for free α-hydroxy-metabolite of midazolam (Greenblatt *et al.*, 1981). Plasma (500 μl) was extracted with 8 ml of ether after alkalinization by adding 50 μl of 1 N NaOH. Seven ml of the organic layer were evaporated to dryness and the residue silylated with BSTFA. The silylated mixture was again evaporated to dryness and the residue taken up in 100 μl of benzene : acetone : ethanol (8:1:1). One or 2 μl of the final mixture was injected into the gas chromatograph equipped with an electron capture detector. The column was a 2 m glass coil, internal diameter 4 mm, packed with 5% OV–101 on a Gaschrom Q support. The limit of detection was 4 ng/ml for midazolam and 3 ng/ml for α-hydroxy-midazolam. The coefficient of variation for replicate analysis was 5% for both compounds.

Urine

The urine aliquots were pooled and assayed for the main urinary metabolite, α-hydroxy-midazolam, after enzymatic deconjugation with glucuronidase-sulphatase. Pooled urine (200 μl) was mixed with 0.8 ml of 0.2 M acetate buffer pH 5.3 and after addition of 20 μl glucuronidase-sulphatase incubated at 37°C overnight. After incubation, urines were brought to pH 12 by adding 0.5 ml of saturated solution of Na_3PO_4 and then extracted with ether.

Pharmacokinetic analysis

The disposition of midazolam can be described by an open two compartment model. Thus concentrations of midazolam in the plasma after intravenous administration were analysed according to the following equation of a two compartment model:

$$Cp = A\,e^{-\alpha t} + B\,e^{-\beta t} \qquad \text{(eqn 1)}$$

where Cp is the total plasma midazolam concentration at time t, A and B are the extrapolated zero intercepts and α and β represent the distribution and elimination rate constants, respectively, using a non-linear least squares fitting programme on a WANG 2200 VP computer system.

The data were weighed with their squared reciprocals. An accurate prediction of plasma concentration profiles was obtained, together with the dimensions of the two compartment model and a precise estimate of the total area under plasma concentration versus time curves (AUC i.v.). Total clearance (CL_{tot}), defined as the constant fraction of the volume of distribution from which the drug is eliminated in unit-time, and expressed in ml/min, was derived from the relationship:

$$Cl_{tot} = \frac{\text{DOSE}}{\text{AUC i.v.}} \qquad \text{(eqn 2)}$$

Concentration versus time data after oral administration of midazolam and of the α-hydroxy-metabolite

were analysed according to the following equation which describes absorption and elimination:

$$Cp = A\,e^{-\alpha(t-t_o)} + B\,e^{-\beta(t-t_o)} + (A+B)\,e^{-Ka(t-t_o)} \quad \text{(eqn 3)}$$

where Ka is the apparent first order absorption rate constant, and t_o is the lag time, included to account for the delay between drug administration and/or its first appearance in the plasma. The computed parameters were used to determine the area under the oral plasma concentration versus time curve (AUC oral), the time at which peak plasma concentrations occurred (T max) and peak plasma concentrations (Cp max). Bioavailability of the oral doses was determined with reference to the intravenous dose by comparison of the respective areas under the concentration versus time curves.

Thus apparent availability was estimated using eqn 4:

$$F = \frac{AUC\,p.o. \times \beta\,p.o. \times X_o\,i.v.}{AUC\,I.V. \times \beta\,i.v. \times X_o\,p.o.} \quad \text{(eqn 4)}$$

The dose X_o administered by both routes is known. The elimination rate constant of the drug is usually readily determinable following both oral and intravenous administration and it is desirable to reduce intrasubject variability in the availability determination by correcting for changes in area which may be due to changes in half-life.

It is very often impossible to obtain a reliable estimate of the apparent volume of distribution of a drug following oral administration, and therefore it must be assumed to be equal to the apparent volume of distribution obtained following intravenous administration.

Results

Plasma levels: intravenous administration

Plasma levels of midazolam and of the free α-hydroxy-metabolite are shown in Tables 1 and 2. A typical concentration versus time curve is shown in Figure 2. After an initial rapid fall in concentration, characterized by the α-distribution constant, the intravenous plot followed a log-linear decline (β-phase). Associated computed kinetic parameters and calculated values for apparent volume of distribution (V_{ss}), AUC i.v. and Cl_{tot} are shown in Table 3. The biological half-life was 2.29 ± 0.42 h. The apparent volume ofdistribution at steady-state (V_{ss}) was 50.2 ± 11.3 l. The total clearance was 323 ± 86 ml/min.

Plasma levels: oral administration

Plasma levels of midazolam and of the free α-hydroxy-metabolite are shown in Tables 1 and 2. A semilogarithmic concentration versus time plot after oral administration of midazolam is also shown in Figure 2. Table 4 illustrates the important parameters

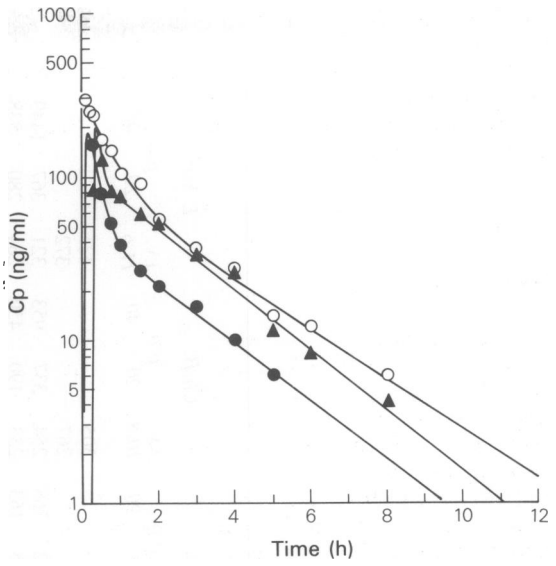

Figure 2 Plasma levels of midazolam following intravenous and oral administration (subject K.M.) O 11.42 mg i.v., ● 10 mg p.o., ▲ 20 mg p.o.

in plasma profile and areas under the curves. Lag time values varied within a range 0–10 min and peak plasma concentrations were 137.8 ± 38.9, 263.8 ± 106 and 1201 ng/ml after 10, 20 and 40 mg, respectively. The rate of absorption was very fast (Ka > 5). Bioavailability results are presented in Table 4.

The levels of the main metabolite in its unconjugated form are fairly high, 40–100% of unchanged drug being reached (Figure 3).

Urinary excretion

The amount of α-hydroxy-midazolam excreted in the urine was determined. Of the administered dose, 60–80% was excreted in the urine as conjugated α-hydroxy-metabolite within 24 h (Table 5). It is interesting to note that urinary excretion is essentially the same after oral and after intravenous administration.

Distribution of the drug between whole blood and plasma

The blood/plasma concentration coefficient (λ) was determined to be 0.53 in normal human blood, thus indicating that the drug is only to a small extent bound to the erythrocytes.

Discussion

Following oral administration midazolam was com-

Table 1 Plasma levels of midazolam (ng/ml) after i.v. and oral administration of midazolam

Time post administration	*Subject and dose (mg)*																	
	R.H.			*K.M.*			*O.A.*			*A.St.*			*Ch.B.*			*E.Sch.*		
	i.v.	*p.o.*		*i.v.*	*p.o.*		*i.v.*	*p.o.*		*i.v.*	*p.o.*		*i.v.*	*p.o.*		*i.v.*	*p.o.*	
	8.2	*10*	*20*	*11.4*	*10*	*20*	*9.6*	*10*	*20*	*9.2*	*10*	*20*	*10.8*	*20*	*40*	*11.6*	*20*	*40*
5 min	291			298			310			314			305			425		
10 min	240			249			281			233			267			372		
15 min	226	52	121	235	157	80	262	170	162	183	142	368	254	337	953	321	367	1449
30 min	172	82	223	169	78	126	220	140	139	183	75	163	233	190	450	264	280	538
45 min	142	59	133	144	51	77	190	101	110	138	53	104	177	135	285	206	197	390
60 min	119	45	100	104	38	73	171	75	99	107	45	98	162	109	259	187	177	315
90 min	83	36	76	89	26	57	138	55	79	69	26	57	124	81	209	142	129	264
2 h	55	23	64	54	21	49	121	43	75	56	19	42	104	78	165	115	102	207
3 h	35	16	52	36	16	32	73	24	59	37	13	25	53	50	105	63	67	126
4 h	24	11	28	27	10	25	53	15	46	23	8	16	47	40	87	48	49	109
5 h	16	6	17	14	6	11	39	12	36	18	6	9	34	25	42	34	29	86
6 h	9	4	11	12	<	8	30	8	26	14	5	7	27	18	34	29	25	69
8 h	4	<	4.5	6	<	4	19	4	14	8	<	4	13	9	19	16	14	47
10 h	<	<	<	6	<	<	10	<	8	5	<	<	7	6	10	15	11	36
12 h	<	<	<	<	<	<	7	<	4	<	<	<	5	4	10	11	13	27

< = Below limit of detection

Table 2 Plasma levels of free α-hydroxy-methyl metabolite (ng/ml) after i.v. and oral administration of midazolam

Time post administration	*Subject and dose (mg)*																	
	R.H.			*K.M.*			*O.A.*			*A.St.*			*Ch.B.*			*E.Sch.*		
	i.v.	*p.o.*		*i.v.*	*p.o.*		*i.v.*	*p.o.*		*i.v.*	*p.o.*		*i.v.*	*p.o.*		*i.v.*	*p.o.*	
	8.2	*10*	*20*	*11.4*	*10*	*20*	*9.6*	*10*	*20*	*9.2*	*10*	*20*	*10.8*	*20*	*40*	*11.6*	*20*	*40*
5 min	6			9			6			17			6			9		
10 min	23			24			17			22			14			20		
15 min	22	19	31	29	68	32	20	60	37	28	80	249	16	136	407	27	125	356
30 min	22	61	182	22	58	93	18	55	70	30	59	185	15	88	236	25	114	180
45 min	19	40	92	18	36	52	16	44	44	22	40	106	13	59	128	24	77	136
60 min	18	27	68	15	24	46	17	38	41	20	37	101	12	40	109	22	69	109
90 min	15	22	47	12	17	45	17	26	35	15	21	62	12	31	88	19	49	90
2 h	12	12	39	9	14	42	16	20	29	13	16	45	11	24	68	18	36	66
3 h	7	11	26	7	12	25	12	10	22	10	10	25	7	15	41	10	20	34
4 h	5	6	16	6	6		9	6.5	17	7	6	17	6	12	32	7	13	28
5 h	4	4	10	5	4	8	6	4.5	13	4	4	8	3	6	14	5	9	18
6 h	<	3	6	3	3	5	5	<	8	3	3	8	3	5	11	4	7	15
8 h	<	<	<	<	<	3	3.5	<	4	<	<	<	<	<	9	3	4	9
10 h	<	<	<	<	<	<	<	<	3	<	<	<	<	<	7	<	<	7
12 h	<	<	<	<	<	<	<	<	<	<	<	<	<	<	3	<	<	3

< = Below limit of detection

Table 3 Computed parameters after intravenous midazolam administration (0.15 mg/kg) to six volunteers

Parameters	*Subjects*						
	R.H.	*K.M.*	*O.A.*	*A.St.*	*Ch.B.*	*E.Sch.*	*Mean ± s.d.*
Dose (mg)	8.19	11.42	9.60	9.15	10.81	11.62	
A (ng/ml)	175	216	163	214	172	293	206 ± 48
α (per h)	1.77	1.52	0.99	1.40	1.22	1.20	1.35 ± 0.27
B (ng/ml)	129	92	147	71	145	128	119 ± 31
β (per h)	0.435	0.347	0.266	0.270	0.300	0.259	0.313 ± 0.068
$t_{1/2}\beta$ (h)	1.60	2.00	2.61	2.56	2.31	2.68	2.29 ± 0.42
AUC (ng/ml h)	397	408	719	414	624	738	550 ± 162
V_{ss} (l)	38.6	59.9	41.9	67.8	47.9	45.0	50.2 ± 11.3
Cl_{tot} (ml/min)	344	466	223	356	289	262	323 ± 86

For explanation of symbols, see text

pletely absorbed. The amount of conjugated α-hydroxy-metabolite excreted in the urine after oral and intravenous administration was practically constant with a very small intra- and interindividual variation. The oral bioavailability ranged from 31 to 72%. The quotient between the AUCs after the high and the low oral dose was considered to be a criterion for linear proportionality between plasma levels and administered doses. The values ranged from 1.78 to 2.56 for the subjects dosed with 10 and 20 mg and from 2.19 to 2.40 for subjects dosed with 20 and 40 mg, respectively. These values demonstrate a linear relationship between plasma levels and the oral doses in the range of 10 and 40 mg. It should be noted that the maximum concentration is attained unusually quickly, i.e., within 15 min. The parallel courses of the plasma level curves of midazolam and the α-hydroxy-metabolite during the terminal phases (Figures 2 and 3) indicate that, in the concentration course of the α-hydroxy-midazolam, the terminal phase is governed by the combined functions of its formation and elimination.

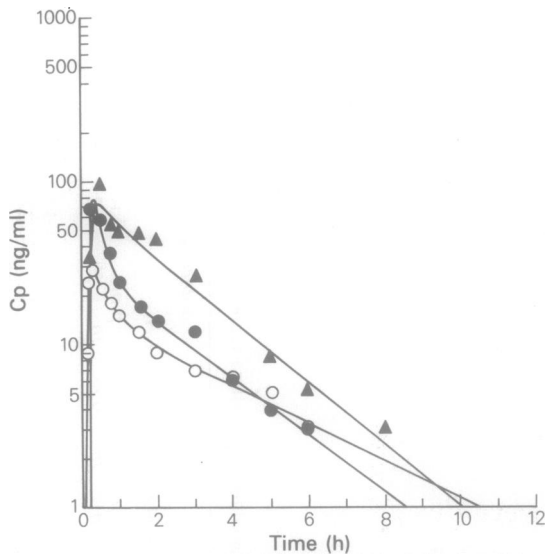

Figure 3 Plasma levels of the α-hydroxy-metabolite following intravenous and oral administration (subject K.M.) of midazolam. Symbols as in Figure 2.

Table 4 Pharmacokinetic characteristics and bioavailability of midazolam tablets

Parameters	*Subjects*						
	R.H.	*K.M.*	*O.A.*	*A.St.*	*Ch.B.*	*E.Sch.*	*Mean ± s.d.*
Dose (mg)	10	10	10	10			10
$t_{1/2}\beta$ (h)	1.50	1.70	2.00	2.00			1.80 ± 0.24
AUC (ng ml h)	141	173	276	162			188 ± 60
F	0.31	0.57	0.48	0.46			0.46 ± 0.11
Dose (mg)	20	20	20	20	20	20	
$t_{1/2}\beta$ (h)	1.60	1.60	2.50	1.60	2.10	2.20	1.90 ± 0.39
AUC (ng ml h)	367	255	466	345	516	670	437 ± 147
F	0.38	0.45	0.32	0.61	0.49	0.64	0.48 ± 0.12
Dose (mg)					40	40	40
$t_{1/2}\beta$ (h)					1.90	2.60	2.0
AUC (ng ml h)					1197	1779	1488
F					0.63	0.72	0.68

For explanation of symbols, see text

Table 5 Excretion of conjugated α-hydroxy-midazolam in urine within 24 h

Variable	*Subjects*						
	R.H.	*K.M.*	*O.A.*	*A.St.*	*Ch.B.*	*E.Sch.*	*Mean ± s.d.*
0.15 mg/kg i.v.							
Amount (mg)	6.3	9.2	6.1	7.0	7.3	8.2	
% Dose	77	81	64	77	68	71	73 ± 6.4
10 mg p.o.							
Amount (mg)	6.8	8.1	7.1	8.0			
% Dose	68	81	71	80			75 ± 6.5
20 mg p.o.							
Amount (mg)	13.3	15.2	14.5	16.1	14.0	12.6	
% Dose	67	76	73	81	70	63	72 ± 6.4
40 mg p.o.							
Amount (mg)					27.7	34.6	
% Dose					69	87	78

The rate of elimination can thus be estimated from the invasion function. On the basis of present information, we can only approximately estimate the elimination half-life of the metabolite to be probably around 1 h. The half-life is to be accurately determined in a study in which the α-hydroxy-metabolite is given intravenously.

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